Quorum Sensing: Alcohols in a Social Situation

Many microbes use extracellular signals to transmit information about population density and environmental conditions. Recent evidence suggests that the budding yeast *Saccharomyces cerevisiae* exhibits this type of regulation and that the signals are aromatic alcohols.

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Microbes often regulate processes that require the coordinated action of multiple cells within a population using extracellular chemical signals [1–3]. Examples of population-dependent microbial activities include virulence towards plants and animals, DNA exchange and the formation of multicellular biofilms. Because these signals generally regulate genes in accordance with high cell density, they have been dubbed 'quorum sensing' molecules [4]. Though the study of quorum-sensing regulation has largely focused on bacteria, density-dependent regulation is emerging as a common trait in eukaryotic microbes as well [3,5]. In a recent paper, Chen and Fink [6] extend the list of microbes that are known to exhibit quorum-sensing to include the model yeast *Saccharomyces cerevisiae*. The newly described quorum-sensing pathway that these authors have uncovered governs filamentation in response to low nitrogen conditions, and the extracellular signals turn out to be the aromatic alcohols tryptophol and phenylethylalcohol.

There are many similarities between the signaling network controled by trypophol and phenylethylalcohol in *S. cerevisiae* (Figure 1) and quorum-sensing pathways previously described in Gram-negative bacteria [1,6]. First, the genes involved in the synthesis of aromatic amino acids are autoregulated and induced upon addition of exogenous signal, suggesting a mechanism for amplifying the signal within the population [6]. Second, while the production of signal is enhanced at high cell densities, it is also affected by environmental conditions, indicating the integrated nature of these signaling pathways [6]. Third, the *S. cerevisiae* quorum-sensing signal controls the formation of pseudohyphae, a trait associated with multicellular processes such as biofilm formation [7] and perhaps, in nature, with virulence towards the grapevine *Vitis vinifera* [8]. Finally, the signal is not general to all ascomycete fungi and may prove to be specific to *S. cerevisiae*. While the dimorphic fungal pathogen *Candida albicans* has been shown to produce tryptophol and phenylethyl alcohol [9], these compounds do not promote the *C. albicans* yeast-to-hyphae transition [6,10] and may in fact repress hyphal development [6]. One might propose that the yeast quorum-sensing pathway may have evolved by co-opting a common catabolic product for the purposes of intercellular signaling.

Many different chemical signals likely participate in the regulation of pseudohyphae formation in *S. cerevisiae* [11,12]. Isoamyl alcohol and 1-butanol, known to contribute to the off-flavors in fermented alcohols, are also derived from amino acid catabolism by *S. cerevisiae* under nitrogen-limiting conditions [13]. Isoamyl alcohol and butanol similarly stimulate pseudohyphal growth in yeast [14,15], but do so through a regulatory cascade that is distinct from that required for the response to tryptophol and isoamyl alcohol [6,15]. Furthermore, ethanol, the primary fermentation

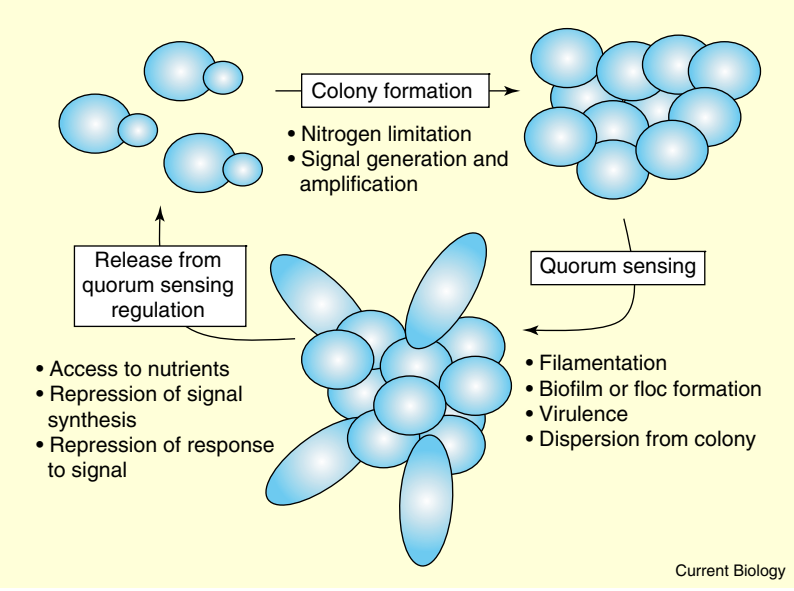

Figure 1. Quorum sensing regulation of pseudohyphae formation in *Saccharomyces cerevisiae*.

This diagram illustrates the integration of cell density-dependent signaling, nutrient availability, and biological response to these cues in *S. cerevisiae*. As individual yeast cells grow to form a colony, the cell density increases and nutrient availability decreases. On nitrogen-poor media, these conditions lead to the induction of genes involved in the synthesis of aromatic amino acids including tryptophol and phenylethyl alcohol. Tryptophol exhibits positive feedback regulation of the transcription of the *ARO* genes necessary for the production of aromatic alcohols. The presence of tryptophol or phenylethylalcohol stimulates pseudohyphal growth by a Tpk2-dependent pathway involving Flo8-mediated transcriptional control of the cell surface protein Flo11. The morphological conversion may promote dispersion, invasion, or multicellularity. The processes involved in release from the effects of aromatic alcohols are less clear. Access to ammonium represses the response to aromatic alcohol signals. Active signal degradation may also occur.

product upon growth on sugars, promotes hyperfilamentation in diploid strains via a MAP kinase-dependent pathway [15]. Together, these data suggest that alcohols serve as important autoregulatory molecules in *S. cerevisiae*. The mechanism for integrating these different cues has yet to be determined. The use of multiple extracellular signals for the purposes of quorum sensing regulation, presumably to 'fine-tune' the outputs of these regulatory cascades, has been well-documented in bacteria such as *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Vibrio harveyi* [2,16].

Because microbes continually need to respond to varying environmental conditions, it is not surprising that quorum-sensing regulation is modulated by physical factors and nutrient availability. In the case of *S. cerevisiae*, quorum-sensing regulation involving aromatic alcohols occurs under low nitrogen conditions, and is repressed upon addition of excess ammonium [6]. Iron availability, oxygen, and carbon source are some of the other environmental conditions that are known to affect the production of quorum sensing molecules in other organisms [16]. Quorum-sensing regulation may in fact aid in adapting to the stress conditions due to pH extremes or nutrient limitation that often exist within dense microbial populations.

There may be several advantages to regulating yeast filamentation in accordance with nitrogen availability and population density (Figure 1). Invasive or filamentous growth may represent a foraging strategy that aids in nutrient acquisition by directing growth away from a dense microbial colony [17]. Similarly, growth as pseudohyphae may allow for the invasion of nutritive surfaces and this effort may require the concerted action of numerous cells [8]. An alternative explanation stems from the fact that the cell-surface Flo glycoproteins that are expressed during filamentation confer hydrophobicity and promote biofilm and aggregate formation [7]. Formation of these multicellular structures may aid in survival in nutrient limiting environments by accessing nutrients that accumulate on surfaces or by retaining nutrients from lysed cells within the population. Future studies may identify additional signals that influence the decision between dispersion and multicellularity under different conditions.

The virulence of many plant and animal fungal pathogens involves the ability to undergo morphological transitions between yeast and filamentous forms. *Candida albicans*, the most common fungal pathogen of humans, forms hyphae in response to chemical inducers and in low nutrient environments, and the signal transduction pathways that govern hyphae formation have many similarities to those that control pseudohyphal growth in *S.cerevisiae* [18]. While *C. albicans* also has quorum-sensing regulation of its morphology, mediated by the sesquiterpene farnesol, the accumulation of the extracellular signal represses, rather than stimulates, filamentous growth at high cell densities [19]. *In vitro* biofilm studies suggest that farnesol production by *C. albicans* may aid in the dispersion of *C. albicans* cells from multicellular biofilms grown on solid surfaces [20].

In summary, our understanding of the molecular basis for multicellular behavior in fungi is in its early stages. The identification of quorum sensing regulation in *S. cerevisiae* opens up the exciting possibility of using one of the most powerful biological model systems available to us today to elucidate the mechanistic details of signal generation and response in fungi, and to understand the fitness benefits of intercellular communication.

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